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In Vitro Antioxidant Evaluation of Extracts of Three Wild Malaysian Plants

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Abstract

The aim of this study is to evaluate the antioxidant activity of three species commonly available in Malaysia. *Mimosa pudica* and *Crotalaria pumila belongs* to family Febaceae while *Lantana camara,* belongs to Verbenaceae. The extracts were prepared by Soxhlet extraction method using ethanol as a solvent. The β-carotene bleaching assay and 1,1-Diphenylpicrylhydrazyl (DPPH) radical scavenging method were used to determine antioxidant properties of plants by measuring decrease in absorbance at 470nm and 517nm to calculate percent inhibition of each specie. The antioxidant activity was then compared with standard Butylated hydroxyanisole (BHA). Among three samples *M. Pudica* showed the highest total antioxidant activity 58% compared with other samples. *Crotalaria* exhibited good antioxidant activity and free radical scavenging activities 54% while *lantana* showed the weaker antioxidant activity 19%.

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Keywords: Antioxidant activity; β-carotene; DPPH; wild species.

1. Introduction

Many plants contain natural antioxidants that act in metabolic response to endogenous production of free radicals and other oxidant species. These responses are due to ecological stress or promoted by toxins produced by pathogenic fungi and bacteria [1]. Recently, interest has increased in naturally-occurring antioxidants that can be used to protect human beings from oxidative stress damage [2]. Over production of reactive oxygen species (ROS) in human beings, by endogenous or external sources, e.g. Tobacco smoke, certain pollutants, organic solvents or pesticides, leads to oxidative stress [3].

Oxygen is an essential for survival however, its univalent reduction generates several harmful reactive oxygen species (ROS), inevitable to living cells and highly associated with the wide range of pathogenesis such as diabetes, liver damage, inflammation, aging, neurological disorders and cancer (4). In spite of comprehensive network of cellular defensive antioxidants, many ROS still escape this surveillance inflicting serious anomalies favoring such diseases states [5]. Though synthetic antioxidants, BHT, BHA and radio protector, war far in are being used widely, however, due to their potential health hazards, they are under strict regulation [6]. Antioxidant principles from natural resources are multifaceted in their multitude/magnitude of activities and provide enormous scope in correcting the imbalance through regular intake of proper

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diet. Therefore, in the recent years, the interest is centered on antioxidants derived from herbal medicine in view of their medicinal benefits [7]. Phyto antioxidants, commonly available, less toxic, serving food and medicinal components have been suggested to reduce threat of wide range of ROS [8].

In the USA, *Crotalaria pumila* Ortega (aerial parts) is used to treat yellow fever and skin rashes. All plant parts of *Crotalaria sessiliflora Vatke., Crotalaria assamica Benth*. and *Crotalaria ferruginea* are being used traditionally in China to treat cancer. Aerial parts of Crotalaria agatiflora Schweinf. are used in Kenya for the treatment of ortitis media, a bacterial infection of ears, as well as for treating sexually transmitted diseases [9].

Mimosa pudica known as chue Mue, is a stout straggling prostrate shrubby plant with the compound leaves which gets sensitive on touching, pinousstipules and globose pinkish flower heads, grows as weed in almost all parts of the country. Leaves and stems of the plant have been reported to contain an alkaloid mimosine, leaves also contain mucilage and root contains tannins. *Mimosa pudica* is used for its anti diarrhoeal [11] anti-convulsant and cytotoxic properties [13]. The plant also contains turgorins, leaves and roots are used in treatment of piles and fistula. Paste of leaves is applied to hydrocele. Cotton impregnated with juice of leaves is used for dressing sinus. Plant is also used in the treatment of sore gum and is used as a blood purifier [5].

Lantana camara, commonly known as wild or red sage is the widest spread species of this genus and regarded both as a notorious weed and a popular ornamental garden plant. It is listed as one of the most important medicinal plant of the world. *Lantana* plant has been reported to possess a number of medicinal properties [5]. Various parts of the plant are used in the treatment of itches, cuts, ulcers, swellings, bilious fever, catarrh, eczema, dysentery and chest complains of the children, fistula, pustules and rheumatism [6]. Some metabolites isolated from their leaves have antitumor activity; antimotality; anti-inflammatory; insecticidal and termicidal effects and antioxidant activity [7].

Hence, in this study we aimed to inspect the total antioxidant by β-carotene bleaching assay and antioxidant activity of ethanolic extracts of *Mimosa pudica, Crotalaria pumila* and *Lantana camara.*

2. Material and Method

2.1 Plant material

All the plants were collected from the residential collage two (KK2) of UMP Gambang campus in February 2012. The leaves of all the three plants are used.

2.2 Preparation of sample

The dried samples were cut into small pieces and ground into fine powder using a dry grinder. The ground samples were sieved to get uniform particle size, then kept it in air tight container and store in a freezer (-20^{O}C) until further analysis.

2.3 Chemicals

All the chemicals used were of analytical grade. 2,2-Diphenyl-1picrylhydrazyl (DPPH), linoleic acid, β-carotene, 2,6-ditert-butyl-4-hydroxyanisole (BHA), solvent ethanol (EtOH) and Tween 40, were supplied from Sigma.

2.4 Extraction of sample

Each ground sample was weighed and transferred into a beaker. Ethanol was added in the round bottom flask and sample was kept in thimble. The heat given to the solvent was just to evaporate it slowly the speed was maintained to one drop per second. It was kept for 5 hours and then crude extract was left on room temperature to dry.

2.5 Measurement of Antioxidant activity

Total antioxidant activity of plant extract was measured according to the method of Velioglu et al. (1998) and Lu and Foo (2000). 1 mg/mL of β- carotene was dissolved in chloroform to make 0.2 mg/mL chloroform solution and pipetted it out into a round bottom flask (50 mL) containing 0.02 mL of linoleic acid and 0.2 mL of 100% Tween 20. The mixture was then evaporated at 40^{O}C for 10 mints by rotary evaporator to remove chloroform. After evaporation, the mixture was immediately diluted with 100 mL of distilled water. The distill water was added slowly to the mixture with vigorous agitation to form an emulsion. Then 5 mL of aliquots of the emulsion was transferred into test tubes containing 0.2 mL of samples in 80% methanol at 1 mg/mL The tubes were then gently mixed and placed at 45 ^{O}C in a water bath for 120 mints. Absorbance of the sample was measured at 470 nm using Spectronic ® Genesys ™ 20 spectrophotometer (Thermo Scientific Company), at initial time (t=0)) against a blank, consisting of an emulsion without β- carotene. Standards at the same concentration with the sample were used for comparison. 0.2 mL of 60% methanol in 5 mL of the above emulsion was used as a control. The measurements were carried out at 20 mints intervals. All determinations were performed at triplicates.

Antioxidant activity was measured in terms of bleaching of β-carotene by using a slightly modified version of formula from Jayaprakasha et al., [11]. According to Jayaprakasha, Singh and Sakariah [11], [8], the time for At and A0 t were at

180 min. In this method, as the absorbance was measured at 120 min, At and A_0t were at 120 min.

$$AA = \left(1 - \frac{(A_0 - A_t)}{(A^{\circ}_0 - A^{\circ}_t)}\right) \times 100 \quad (1)$$

Where A_0 and A^{O}_0 are the absorbance values measured at initial time of the incubation for samples and control respectively, while At and A½t are the absorbance values measured in the samples or standards and control at t = 120 min.

2.6 Free radical scavenging assay

Effect of plant crude extracts on DPPH radical was measured based on the method modified by Lu & Foo (2000) and Lai, Chou & Chao (2001). An aliquot of 200 µl of plant extract (0.62 - 4.96 mg/ml), BHA (0.04 - 1.28 mg/ml) were mixed with 800 µl of 100 mM Tris - HCl buffer (pH 7.4). The mixture was then added to 1 ml of 500 µM DPPH. This was made up to a DPPH final concentration of 250 µM. The mixture was shaken vigorously and left to stand at room temperature for 20 min in a dark room. Absorbance at 517 nm was measured using a UV-Vis spectrophotometer until the reading reached a plateau. The capability of plant extracts to scavenge the DPPH radical was calculated by using the following equation:

Scavenging effect (%) :

$$1 - \frac{\textit{(Absorbance of sample at 517 nm)}}{\textit{(Absorbance of control at 517 nm)}} X\,100 \quad (2)$$

EC50 value was determined from the plotted graph of were carried out and their activity was calculated by the percentage of DPPH scavenged.

3. Result

Figure 1 shows scavenging activity calculated by measuring absorbance versus the concentration of plant extracts, which is defined as the amount of antioxidant necessary to decrease the initial DPPH radical concentration by 50%. *Mimosa, pudica*, *crotolaria* and *lantana* ethanolic extracts showed mean total antioxidant activity of 58%, 54%, 19%, respectively, while the standarard (BHA) showed 68% activity (Figure 1).

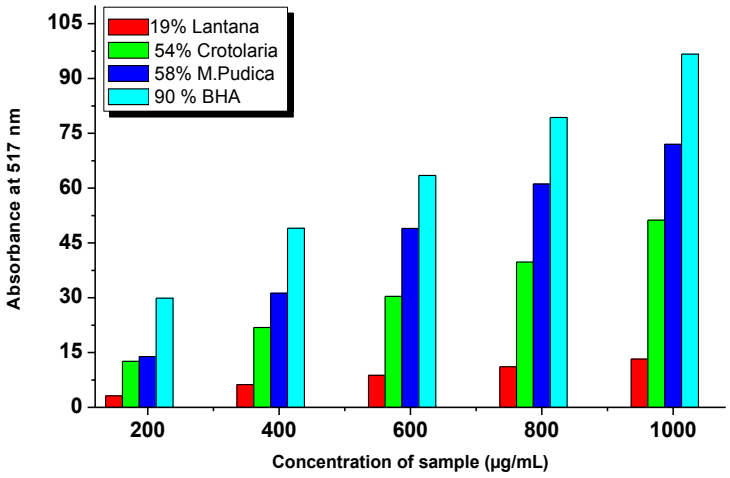


Fig. 1. Scavenging activity of ethanolic extract of *Mimosa pudica, Crotolaria* and *lantana* on DPPH radical

Triplicate measurements in "Figures 2" show the comparative β-carotene bleaching rates of the control, standard and plant extracts. It shows a decrease in absorbance of β-carotene in the presence of different plant extracts due to the oxidation of β-carotene and linoleic acid. This indicates that all tested extracts possessed antioxidant capacity.

The highest antioxidant activity among the plants was observed in *Mimosa Pudica* while *Lantana* showed very low

antioxidant activity. There was a significant difference ($p<0.05$) between the means of total antioxidant activity among the ethanolic extracts of the samples.

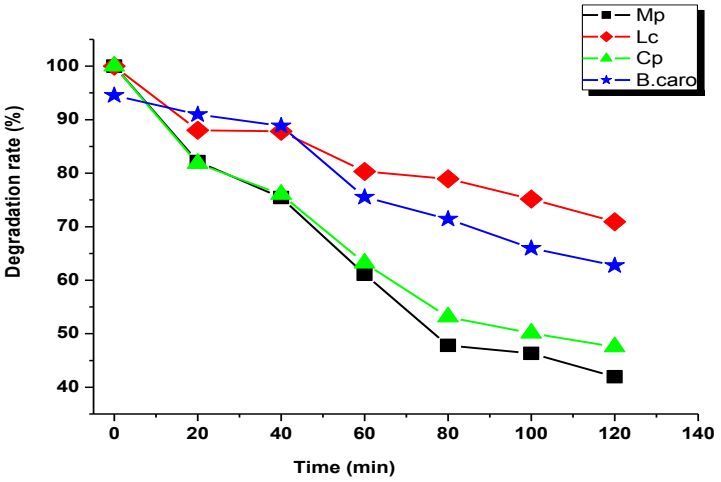


Fig. 2. Mean total antioxidant activity of plant ethanolic extract and BHA. Antioxidant activity was measured using a β-carotene bleaching assay. Whereas Mp is Mimosa pudica, Lc is Lantana cemara, Cp is Crotolaria pumila and B-Caro is stands for beta carotene.

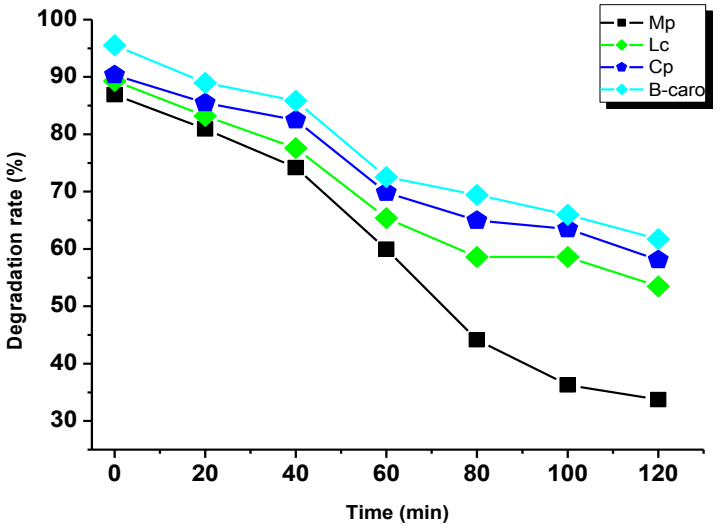
4 Discussion

According to Jayaprakasha et al. [11], the bleaching mechanism of β-carotene is a free radical mediated phenomenon resulting from the formation of hydroperoxides from linoleic acid. In the absence of antioxidant, β-carotene will undergo rapid discoloration. Linoleic acid will become a free radical with a hydrogen atom abstracted from one of its diallylic methylene groups. The radical formed then attacks the highly unsaturated β-carotene molecules. When β-carotene molecules lose their double bonds by oxidation, the compound loses its chromophore and orange colour, which can be monitored spectrophotometrically. The presence of antioxidants in the different extracts can protect the extent of β-carotene bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system.

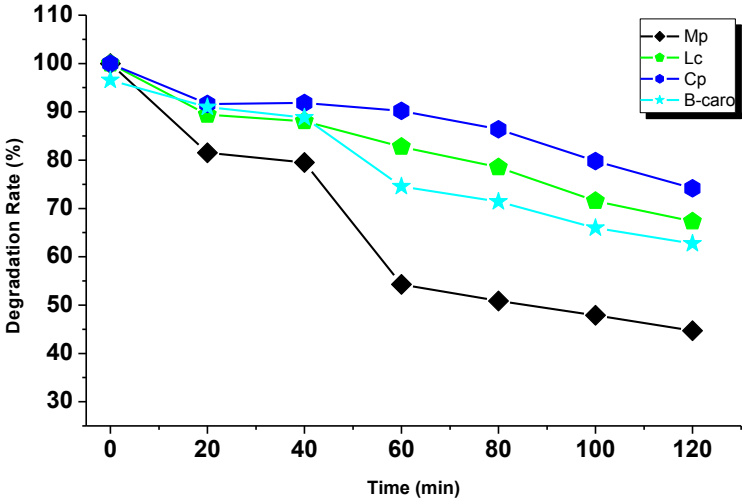
In order to provide additional data on antioxidant potential of the ability of the plant extracts to act as free radical scavengers or hydrogen donors, DPPH radical scavenging activity assay was carried out.

This study showed different plant extracts of the studied samples to show varying degrees of free radical scavenging activity. DPPH radical scavenging activity was seen to increase as the concentration increased from 0.32 to 1.20 mg/ml for all samples and 0.05 to 1.25 mg/ml for standards. These findings are in agreement with the results obtained by Lu & Foo [12].

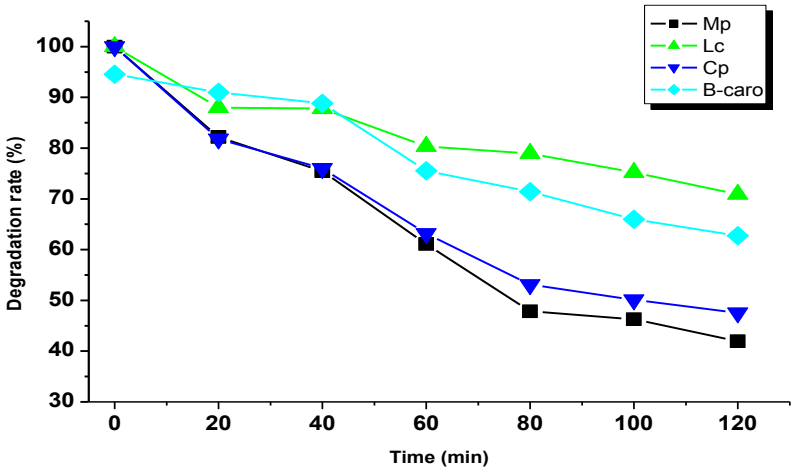
These results also supported by the study of Duffy & Power [13] who described different samples in different solvents to give different antioxidant potentials. Previous studies reported that ethanolic extracts of licorice samples displayed high antioxidant potential compared to water extracts. However, other ethanolic Chinese plant extracts showed little antioxidant potential [13].



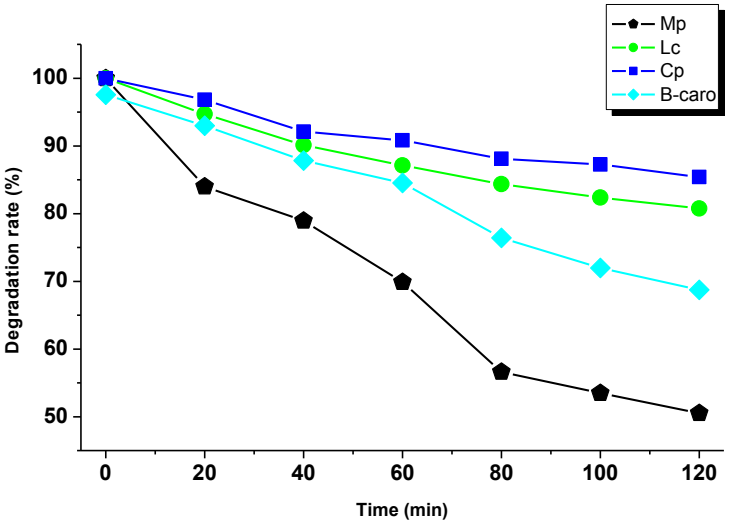
a. **100ppm**



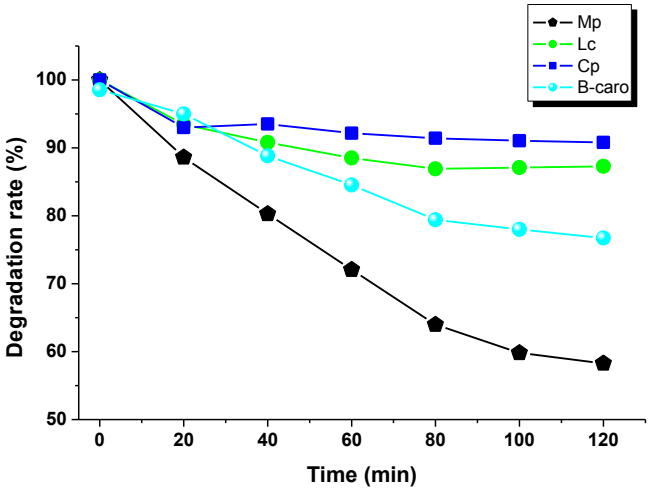
b. **250ppm**



c. 500ppm



d. 750ppm



e. **1000ppm**

Fig. 3(a-e). Comparing the change in the absorbance with the passage of time in various concentration of three plant samples in β-carotene bleaching assay. Whereas Mp is Mimosa pudica, Lc is Lantana cemara, Cp is Crotolaria pumila and B-Caro is stands for beta carotene.

The results obtained on total antioxidant activity of ethanolic extract were supported by the findings from DPPH radical scavenging activity. This finding is in agreement with the previous results reported by Lu & Foo [12] where flavonoids from apple showed the highest antioxidant activity using β-carotene bleaching method and DPPH scavenging assay. The difference in the DPPH radical scavenging activity of each sample in different extracts implies that the extracting solvent used would affect the radical scavenging potency.

5. Conclusion

This study showed that *Mimosa Pudica, Crotolaria* and *Lantana* possessed varying degrees of antioxidative activity in alcholic mediums. Ethanolic extracts of *Crotolaria* and *Lantana* exhibited lower antioxidant activity based on total antioxidant and free radical scavenging activities. *Mimosa Pudica* possessed the highest antioxidant activity.

Although their antioxidant activity was lower than that of commercial antioxidants (BHA), other potential benefits of plants which can contribute to human health should be explored in future studies.

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